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(54) Title: TRANSDERMAL TRANSPORT OF MOLECULES

(57) Abstract

The present invention comprises compositions and methods that are transdermal transport of molecules, including macromolecules. The present invention also includes compositions and methods that are useful for transdermal transport of molecules to treat various conditions of a patient.

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TRANSDERMAL TRANSPORT OF MOLECULES

PRIOR RELATED APPLICATION

The present application is a continuation-in-part of pending United States patent application serial number 08/685,235 filed on July 23, 1996.

TECHNICAL FIELD

The present invention is related to a composition and a method for topical application of a composition that facilitates transdermal delivery of molecules. More particularly, the present invention is related to the transdermal delivery of molecules including macromolecules.

BACKGROUND OF THE INVENTION

Delivery of molecules to desired locations in effective amounts has presented a continuing challenge to health care professionals. Conventional delivery methods include oral administration, administration by suppository or injection, and administration into the cerebrospinal fluid. However, many of these methods involve potentially serious side effects such as infection, hematomas, and damage to nervous and muscular tissue. In addition, these conventional methods often require the participation of a health care professional and involve scheduling a visit and traveling to a medical office. Other problems affect efficacious delivery of

molecules using these methods. Molecules may be metabolized in the liver and gastrointestinal system, or excreted through the kidneys before reaching the desired site of action at the desired concentration thereby necessitating administration of higher concentrations of the active ingredients. Furthermore, some conditions and diseases do not respond to orally administered molecules and formulations.

Previous attempts to administer molecules through the stratum corneum of the skin have generally been limited by the size of the molecule since the stratum corneum provides an effective barrier to penetration. Currently, lipophilic molecules such as steroids and very small molecules such as nicotine have been administered through the skin. What is needed is a composition and method for transdermally delivering molecules of desired size and characteristics through the stratum corneum in close proximity to the desired site of action so that effective concentrations may be attained with minimal deleterious side effects or degradation in the gastrointestinal system.

For example, many patients with localized pain due to arthritis, bursitis, sprain or muscle strain, bruises or hematomas cannot tolerate conventional nonsteroidal anti-inflammatory drugs, commonly known as NSAIDS. In addition, topical administration of conventional NSAIDS has largely been ineffective because only a therapeutically ineffective amount of the drug can penetrate the skin. In addition, conditions such as acne, psoriasis and eczema are typically refractory to topical or oral administration of NSAIDS.

Bromelain is a protease composition that is isolated from pineapple. The composition has been reported to have anti-inflammatory activity when administered orally or parenterally (see Taussig, S.J., "The mechanism of the physiological action of Bromelain", *Medical Hypotheses*, 6; 99-104, 1980). Commercially available bromelain used in the

manufacture of pharmaceuticals is not a chemically homogeneous substance, but the principal component is a proteolytic enzyme that is a glycoprotein. The molecular weight of bromelain is approximately 33,000 Daltons.

Capsaicin is an oleoresin obtained by extracting cayenne pepper with ether. The synthetic capsaicin is trans-8-methyl-vanillyl-6-nonenamide. Capsaicin gels have been described in U.S. Patent No. 5,178,879 as being effective in treating topical pain. In addition, capsaicin is available commercially in over-the-counter compositions intended for pain relief including lotions such as HEET, OMEGA OIL, SLOAN'S LINIMENT and ZOSTRIX.

Bromelain has been reported to be an anti-inflammatory agent, an inhibitor of platelet aggregation, an agent that increases proteolytic and fibrinolytic activity in blood, and a selective prostaglandin inhibitor. Bromelain has been administered by injection and has been reported to be effective after oral administration. However, because bromelain is a relatively large molecule, it cannot be administered transdermally using prior art formulations. Accordingly, what is needed in this example is a composition that will effectively transport molecules, such as bromelain, that are effective in treating a wide variety of inflammatory conditions by topical application of the composition.

Other conditions such as autoimmune diseases that affect the connective tissue and joints could be treated by transdermal delivery of molecules. Alleviation of the pain associated with these conditions and inhibition of inflammatory processes would be of great benefit to these patients.

What is needed is a composition and method for transdermally delivering molecules of desired size and characteristics through the stratum corneum so that effective concentrations may be attained with minimal deleterious side effects or degradation in the gastrointestinal system.

BRIEF SUMMARY OF THE INVENTION

The present invention addresses the needs in the prior art by providing a composition and method for delivering molecules of desired size through the stratum corneum and into the dermis, the hypodermis, or adjacent connective tissue and joints. The present invention also provides a composition and method of transdermal delivery into the bloodstream for systemic action. The present invention is particularly useful for delivering molecules of desired size into the germinal epithelium and nail bed. The present invention provides novel compositions of penetrating agents that permit transdermal delivery of molecules, including macromolecules such as enzymes, and immunoglobulins, after simple topical application to the skin. This novel approach provides a method for treating a variety of conditions without the need for injection or oral administration of molecules.

The present invention addresses the needs in the prior art by providing, in one embodiment, an anti-inflammatory composition containing a proteolytic enzyme, preferably bromelain, and capsaicin. The anti-inflammatory composition is capable of being administered transdermally to a human or animal. The present invention is also a topical anti-inflammatory composition comprising an effective amount of bromelain, an effective amount of capsaicin, and a pharmaceutically effective penetrating agent. Preferred penetrating agents are effective amounts of lecithin organogel, phospholipid gels, and/or poloxamer organogel which are optionally combined with *n*-decylmethyl sulfoxide. The preferred penetration enhancers are isopropyl palmitate, isopropyl myristate, poloxamer phospholipid gel, ethanol, n-decylmethylsulfoxide [NDMS], and ethoxydiglycol.

In one embodiment, the present invention provides an easy and safe method of administering an effective anti-inflammatory macromolecule that, in the prior art, could

only be administered parenterally or orally. By administering the composition topically, directly at the site of the inflammation, the present invention provides a more effective means of treating the inflammation. The present invention is useful for treating a variety of inflammatory conditions which may be painful conditions including neuralgia, myalgia, rheumatoid arthritis, osteoarthritis, sprains, strains, bursitis, myositis, tendonitis, carpal tunnel syndrome, chondromalacia, eczema, inflammation due to infections by microorganisms, bites, acne, dermatitis, thrombi, phlebitis, hematoma, atopy, psoriasis, and integumental pain.

The present invention provides a composition and method for the treatment of various conditions, including but not limited to conditions wherein the condition is pain, or deficiencies or imbalances of the integumentary system, immune system, endocrine system, reproductive system, cardiovascular system, musculoskeletal system, nervous system, digestive system, and respiratory system.

The present invention provides a composition and method for the treatment of various conditions, including but not limited to fungal conditions. Such fungal conditions include, but are not limited to conditions such as onchomycosis and other fungal diseases of the skin and scalp.

The present invention provides a composition and method for the treatment of the effects of sun exposure on the skin, including the condition of solar elastosis.

In addition, the present invention provides an alternative composition and method to conventional NSAIDS for treating inflammation. The present composition does not have the side effects that are often seen with NSAIDS. Some of these side effects include, but are not limited to, somnolence, confusion, gastric upset, gastrointestinal bleeding, chondrocyte dysfunction and kidney damage.

In one embodiment, the composition of the present invention comprises bromelain and capsaicin in a

pharmaceutically acceptable penetrating composition optionally containing *n*-decylmethyl sulfoxide or cholesterol. The present invention can include other pharmaceutically acceptable components such as gelling agents, compounding agents, scents and the like. In especially preferred formulations, the composition further contains a lecithin organogel. In other preferred formulations, the composition further contains phospholipids such as phosphatidylcholine, also known as lecithin, phospholipid gels, or a poloxamer organogel. The combination of a transdermal formulation of bromelain and capsaicin act synergistically to reduce pain. The composition of the present invention can also include other pharmaceutically active agents such as antibacterial, anticancer, antihelminthic, antifungal, antiprotozoal or antiviral agents.

The present invention also includes methods for topically treating inflammation due to a wide variety of causes comprising the step of topically administering a therapeutically effective amount of a composition comprising bromelain and capsaicin in a pharmaceutically acceptable vehicle. The vehicle may include lecithin organogel, phospholipids, poloxamer lecithin organogel, optionally combined with *n*-decylmethyl sulfoxide or cholesterol, and/or ethanol.

Accordingly, it is an object of the present invention to provide a composition and method for facilitating transdermal transport of large molecules.

Another object of the present invention is to provide a composition and method for facilitating transdermal transport of molecules. Included in this category are molecules as large as 500,000 Daltons as well as dimers, trimers and tetramers of these molecules, although the present invention encompasses transport of larger molecules and constructs such as plasmids. The molecules include, but are not limited to the following; enzymes including but not limited to antiinflammatory enzymes, proteolytic enzymes and

elastase, enzyme inhibitors, including but not limited to protease inhibitors, coenzymes, proteins, receptors, peptides, amino acids, hormones, hormone agonists and antagonists, interleukins, immunoglobulins, antibodies, cytokines, monokines, immunosuppressants, immunomodulators, drugs, growth factors including epidermal growth factor, vitamins, plant extracts, lipids, plasmids, nucleic acids, including but not limited to ribonucleic acids and deoxyribonucleic acids, nucleotides, neurotransmitters, neurotransmitter receptor agonists and antagonists, mitotic inhibitors, steroids, lipids, fatty acids, and carbohydrates.

Other molecules and compounds that may be transported with the composition and method of the present invention include, but are not limited to the following: skin moisturizers (for example alpha hydroxyacids, etc.), ultraviolet blockers, protease inhibitors, inhibitors of pain transmission, vasodilators, vasoconstrictors, antibiotics, antifungal drugs, antiprotozoal drugs, antihelminthic drugs, antibacterial drugs, antiviral agents, and anticancer drugs, including drugs that affect nucleotides and DNA replication, drugs that affect transcription and/or translation, inhibitors of signal transduction systems, and stimulators of signal transduction systems.

It is another object of the present invention to provide a composition and method for topically treating conditions in patients.

Another object of the present invention is to provide a composition and method for topically treating painful conditions in patients.

Yet another object of the present invention to provide a composition and method for topically treating inflammatory conditions.

It is another object of the present invention to provide a composition and method for topically treating arthritis.

It is yet another object of the present invention to provide a composition and also a method for topically treating bursitis.

It is yet another object of the present invention to provide a composition and method for treating acne, psoriasis and eczema.

It is yet another object of the present invention to provide a composition and method for treating flea allergy, insect bites, dermatitis, thrombi, phlebitis, hematoma, and atopy.

It is yet another object of the present invention to provide a composition and method for topically treating inflammation that is safe and effective and does not have the side effects of conventional NSAIDS.

Another object of the present invention to provide a composition and method for topically treating fungal conditions.

Yet another object of the present invention is to provide a composition and method for treating solar elastosis.

It is a specific object of the present invention to provide a composition and method for the topical treatment of fungal disease of the skin and scalp.

It is another specific object of the present invention to provide a composition and method for the topical treatment of onchomycosis of the toenail and fingernail.

These and other objects, features and advantages of the present invention will become apparent after a review of the following detailed description of the disclosed embodiments and the appended claims.

DETAILED DESCRIPTION

The present invention provides a composition comprising a pharmaceutically effective penetrating agent, and a method for transdermally administering molecules. The composition and method or the present invention may be used

to treat numerous conditions by transdermally delivering desired molecules. The term "condition" means any biological state of a patient. Conditions may include numerous biological states including, but not limited to, deficiencies or imbalances of the following systems: immune, endocrine, reproductive, integumentary, cardiovascular, musculoskeletal, nervous, digestive, and respiratory. Conditions include other biological states including, but not limited to, painful conditions, inflammatory conditions, fungal conditions including fungal disease of the skin and scalp, and specifically onchomycosis.

Conditions may include biological states of different tissues including, but not limited to, the following: connective, muscle, nervous, skeletal, lymphoreticular, cutaneous, endocrine, and exocrine. Conditions may also refer to the need for a particular treatment with medicines, such as a vaccination, a hormone supplementation, an anti-inflammatory regimen, an antifungal regimen, and/or pain medication.

The term "patient", as used herein, means any human or animal. The term "pharmaceutically effective penetrating agent" means *n*-decylmethyl sulfoxide (NDMS), lecithin organogel, phospholipids gels, cholesterol with or without ethanol, ethoxy diglycol, poloxamer organogel, poloxamer phospholipid gel and poloxamer organogels in combination with phospholipids. The term "an effective amount of a pharmaceutically effective penetrating agent" means that amount of the pharmaceutically effective penetrating agent that is capable of transdermally transporting a molecule. More specifically, in the case of onchomycosis, the term "an effective amount of a pharmaceutically effective penetrating agent" means that the pharmaceutically effective penetrating agent is capable of bringing therapeutic levels of antifungal to the germinal epithelium as well as to the nail bed.

The term "topical administration" means application to the surface of the skin. Topical administration may take many forms including, but not limited to, gels,

creams, sprays, rinses, ointments, salves, balms, liposomes, time release vehicles, micelles, and skin patches or other forms of pads.

The term "phospholipids" is used to mean water-insoluble biomolecules that are highly soluble in organic solvents. Phospholipids are generally derived from glycerol and consist of a glycerol backbone, two fatty acid chains, and a phosphorylated alcohol. Phospholipids, either singularly, or in combination with other phospholipids can form gels capable of transdermal delivery. As utilized in this invention, phospholipids can be used individually and do not have to be combined with other components such as lecithin organogel or poloxamer organogel. In another embodiment of the present invention, phospholipids may be used in combination with lecithin organogel and/or poloxamer organogel. Phospholipids may also be optionally combined with n-decylmethyl sulfoxide (NDMS), ethoxy diglycol, cholesterol and/or ethanol.

A preferred phospholipid for use in the present invention is phosphatidylcholine, also known as lecithin. Stedman's medical dictionary [21st ed., page 879] defines lecithin as any of a group of phospholipids which upon hydrolysis yield two fatty acid molecules and a molecule each of glycerophosphoric acid and choline. There are several varieties of lecithin. Lecithin is a mixture of the diglycerides of stearic, palmitic, and oleic acids, linked to the choline ester of phosphoric acid. Soybean lecithin is a preferred lecithin and may contain the following acids; palmitic, stearic, palmitoleic, oleic, linoleic, linolenic and arachidonic. In some lecithins both fatty acids are saturated while others contain only unsaturated fatty acids for example, oleic, linoleic or arachidonic. In other lecithins one fatty acid is saturated, the other unsaturated. Lecithins are found in nervous tissue, cardiac tissue, in egg yolks and in soy which constitutes the most common and economical source of phosphatidylcholine.

It is therefore to be understood that any reference herein to lecithin or phosphatidylcholine is intended to include any combination of lecithin-like phospholipid compounds as is well known in the art. Examples of other phospholipids which can be used in accordance with the present invention include phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, and phosphatidic acid. A mixture of any of the above phospholipids may be also be used in the present invention.

The term "PLURONIC" refers to poloxamer compounds and are sold collectively under the trademark PLURONIC (BASF, Parsippany, NJ). PLURONIC F-127 (PL 127) corresponds to poloxamer 407, a polyoxypropylene-polyoxyethylene block copolymer described by Schmolka in the *Journal of Biomedical Materials Research* 6:571-582, 1972. Other PLURONICS may be used in the present invention. As used in this application, the terms PLURONIC organogel, poloxamer organogel, and polyoxyethylene/polyoxypropylene organogel are synonymous. As used in this application, the term PLURONIC phospholipid gel and poloxamer phospholipid gel are synonymous.

The "enhanced penetration" caused by compositions of this invention as used in topical application with this method, means increased penetration into the skin, and is achieved with compounds such as lecithin organogel, poloxamer organogel, phospholipid gels or poloxamer phospholipid gels including but not limited to phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, and phosphatidic acid and phosphatidylcholine optionally combined with n-decylmethyl sulfoxide (NDMS), PLURONIC F127, ethoxy diglycol, ethanol, or cholesterol. Enhanced penetration can be observed in many ways known to those skilled in the art.

The present invention is a composition and method for transdermally transporting molecules to treat

conditions in patients. As defined above, these conditions include numerous biological states. The pharmaceutically effective penetrating agents of the present invention transport different molecules across the stratum corneum and into the lower layers of the epidermis, into the dermis, the hypodermis and into the bloodstream, lymphatic circulation, and adjacent connective tissue and joints. The pharmaceutically effective penetrating agents of the present invention facilitate access of the molecules to the nervous system. In this manner, for example, transdermally transported molecules, such as calcitonin gene-related peptide, inhibitors of substance P, opiate agonists or various types of anesthesia may affect peripheral nerve endings for modulation of pain transmission in primary sensory afferents. The pharmaceutically effective penetrating agents of the present invention also facilitate access of therapeutic substances, such as antifungal agents, to the germinal level as well as to the nail bed.

The present invention is a composition and method for topically treating conditions such as inflammation caused by a wide variety of causes including, but not limited to, arthritis, bursitis, phlebitis, sprains, muscle strains, bruises, phlebitis, flea allergy, bites, phlebitis, atopy, and hematomas. In one embodiment, the present invention is a composition containing bromelain and capsaicin. The composition further comprises lecithin organogel, and/or poloxamer organogel and/or phospholipids optionally combined with NDMS or cholesterol, as agents to increase the transport of molecules across the skin.

In one embodiment, proteolytic enzymes can be transported through the use of the present invention, including but not limited to, bromelain, collagenase, gelatinase, trypsin, chymotrypsin, papain and elastase. In one embodiment, the present invention comprises bromelain (PCAA, Kinghurst, Houston, TX) at a concentration of between approximately 0.5% and 40% by weight, with a preferred concentration of

between approximately 3% and 25% by weight, with the most preferred concentration of approximately 7.5% by weight.

The anti-inflammatory composition of the present invention also optionally contains capsaicin. The capsaicin can be either naturally occurring capsaicin oleo resin (PCAA, Kinghurst, Houston, TX) which is commonly extracted from cayenne pepper with an organic solvent such as ether or can be synthetic capsaicin, trans-8-methyl-vanillyl-6-nonenamide (PCAA Kinghurst, Houston, TX). The capsaicin is present in the transdermal anti-inflammatory of the present invention at a concentration of between approximately 0.01% and 0.5% by weight, with a preferred concentration of between approximately 0.1% and 0.35% by weight, with the most preferred concentration of approximately 0.25% by weight. Other molecules which affect pain transmission are considered within the scope of this invention. Some of these molecules include but are not limited to, anesthetics, analgesics, ganglionic blockers, receptor blockers, receptor antagonists and agonists, enzymes and enzyme inhibitors, catecholamines, indoleamines such as histamine, serotonin and related analogs, molecules that affect neurotransmitter reuptake mechanisms, peptides and peptide analogs such as those related to calcitonin gene-related peptide, substance P, bradykinin, opiate agonists and antagonists, and steroids.

The present invention also includes a pharmaceutically acceptable penetrating agent. A preferred penetrating agent is NDMS used in combination with lecithin organogel, phospholipid gels, poloxamer organogel, and poloxamer organogel combined with phospholipid gels. NDMS has been described as an agent that is useful in facilitating the delivery of small molecules transdermally (see Hoo-Kyun, C., *et al.* "Transdermal delivery of bioactive peptides: The Effect of *n*-Decylmethyl Sulfoxide, pH, and Inhibitors on Enkephalin Metabolism and Transport", *Pharm. Res.*, Vol. 7, No. 11, pgs. 1099-1106, 1990; Hoo-Kyun, C., *et*

al. "Some General Influences of n-Decylmethyl Sulfoxide on the Permeation of Drugs Across Hairless Mouse Skin", *Soc. Invest. Derm.*, Vol. 96, pgs. 822-826, 1991; and Smith, E.W., Maibach, H. eds., Percutaneous Penetration Enhancers, CRC Press, pg. 109, 1995; which are incorporated herein by reference). However, there is nothing in the literature known to the inventor that would indicate that NDMS alone would be effective in facilitating the transport of macromolecules, such as bromelain, across the skin. NDMS must be used in conjunction with poloxamer organogel, phospholipids, or lecithin organogel. Although not wanting to be bound by this statement, it is believed that NDMS enhances permeation of the skin by affecting the barrier nature of the stratum corneum.

NDMS (PCAA Kinghurst, Houston, TX) is present in the transdermal anti-inflammatory composition of the present invention at a concentration of between approximately 0.1% and 1% by weight, with a preferred concentration of between approximately 0.15% and 0.8% by weight, with the most preferred concentration of approximately 0.5% by weight. NDMS is dissolved in 10 mL of a 75% solution of ethanol. Finally, purified water is added. Ethanol (98%) may also be used to dissolve lecithin and then either boiled off completely or partially to leave a final ethanol concentration of 3% to 8.5%. While not wanting to be bound by the following statement, it is believed that 3% to 8.5% ethanol may enhance penetration.

Another preferred penetrating agent and delivery vehicle is lecithin organogel which is a combination of lecithin, isopropyl palmitate, or isopropyl myristate and/or ethanol and water. Lecithin organogels have been described as vehicles that are useful in facilitating the delivery of low molecular weight compounds transdermally (Willimann, H., et al., "Lecithin Organogel as Matrix for Transdermal Transport of Drugs", *J. Pharm. Sci.*, Vol. 81, 1992, which is incorporated herein by reference). The lecithin organogels are obtained by

adding small amounts of water to a solution of lecithin in organic solvents and/or ethanol. Generally, lecithin organogels are prepared at room temperature by first dissolving lecithin in an organic solvent such as isopropyl palmitate or isopropyl myristate and then adding enough water while stirring to obtain the desired gel. Lecithin used in the gel preparations of the present invention generally contain at least 95% phosphatidylcholine.

Solvents used in the preparation of a variety of gels, including lecithin gels, all of which are appropriate in practicing the present invention, are described in Scartazzini, et al. *Journal of Physical Chemistry* 92:829-833, 1988, and Luisi, P.L. et al. *Colloid and Polymer Science* 268:356-374, 1990, both of which are incorporated herein by reference in their entirety. Specifically these solvents include the following: ethyl laurate, butyl laurate, ethyl myristate, isopropyl myristate, isopropyl palmitate, isooctane, cyclooctane, cyclododecane, methyl cyclohexane, tert-butylcyclohexane, phenylcyclohexane, bicyclohexyl, 1,3,5-triisopropylbenzene, octylbenzene, trans-decalin, (1R)-(+)-trans-pinane, (1R)-(+)-cis-pinane, n-pentane, n-hexane, n-heptane, n-octane, n-nonane, n-decane, n-undecane, n-dodecane, n-tridecane, n-tetradecane, n-pentadecane, n-hexadecane, n-heptadecane, 2,3-dimethylbutene, 1-hexene, 1,7-octadiene, tripropyllamine, tributylamine, triisobutylamine, trioctylamine, dibutyl ether and 2-dodecen-1-yl succinic anhydride.

In addition to isopropyl palmitate and isopropyl myristate, other solvents may be used in the practice of the present invention. These solvents include, but are not limited to the following: mineral spirits, kerosene, isooctane, petroleum ether, diethyl ether, benzene, toluene, methanol, ethanol, heptanol, methyl isobutyl ketone, cyclohexanone, methylene chloride, choloroform, chlorodifluoromethane, tetrahydrofuran, oleyoleate, 2-octyldodecanol, cetyl and

stearyl 2-ethylhexanoate, n-octanol, ethyl laurate, isooctane, cyclopentane, cyclohexane, and cycloheptane.

In a preferred embodiment, lecithin organogel may be made from PHOSPHOLIPON 90 (American Lecithin Co., Oxford, CT). In this embodiment, lecithin organogel comprises 1:1 to 1.5:5 (weight/vol) of PHOSPHOLIPON 90 to isopropyl palmitate. Water is added to form the desired gel. Other penetrating agents including, but not limited to cholesterol (2% to 100%) with a preferred range of cholesterol to PHOSPHOLIPON 90 of 3:7 to 3:10. These ingredients are combined with sufficient ethanol to solubilize the mixture. Ethanol is subsequently evaporated, leaving a complex of cholesterol:PHOSPHOLIPON 90. Alternatively, 3.5% - 8% ethanol may be retained in the complex to enhance penetration.

Willimann et al., *Journal of Pharmaceutical Sciences* 81:871-874, 1992, examined the efficacy of lecithin organogels for use in the transdermal delivery of drugs such as scopolamine and broxaterol. Willimann et al., also observed that lecithin organogels had no detrimental effect on skin when compared to control samples treated with physiological saline (see page 872, *Journal of Pharmaceutical Sciences* 81:871-874, 1992).

It is to be understood that the soy lecithin of the present invention is a preferred lecithin source and a preferred source of phosphatidylcholine. However it is to be understood that lecithin may be obtained from other sources. Lecithin and may be dissolved in isopropyl palmitate or isopropyl myristate (gm/gm) to achieve a final concentration in the composition of from approximately 10% - 98%, with a more preferred final concentration of from approximately 20% - 50%. Lecithin at 98% is dissolved gram per gram of isopropyl palmitate to yield a 49% solution of lecithin in isopropyl palmitate. Lecithins may optionally be derived from eggs, and organs such as heart, brain, and liver, and used at concentrations of

approximately 10% - 100%, with more preferred final concentrations of from approximately 10% - 50% when dissolved in isopropyl palmitate, isopropyl myristate or ethanol. When PLURONIC F-127 or another poloxamer is not used in combination with lecithin organogel, a range of final concentrations of lecithin in the organogel is about 0.5% - 98% and the amount of water in the composition is varied accordingly. For example, 95% phosphatidylcholine dissolved in equal amounts of isopropyl palmitate or isopropyl myristate may be diluted with 10% to 30% water to produce a final phosphatidylcholine concentration of from 12.5% to 32.5%. Alternatively, 100% phosphatidylcholine may be dissolved 1:1 in isopropyl palmitate or isopropyl myristate followed by dilution with 10% to 30% water to produce a final phosphatidylcholine concentration of from 20% to 40%. In yet another embodiment, 100% phosphatidylcholine may be dissolved in 98% ethanol which is then boiled off before addition of 10% to 30% water to produce a final phosphatidylcholine concentration of 70% to 90%. The use of PLURONIC 127 will further reduce the phosphatidylcholine content of the gel by a factor of 3 to 4 depending on the amount of PLURONIC 127 added to the mixture.

Another penetrating agent of the present invention includes lecithin dissolved in isopropyl palmitate or another solvent in combination with a final concentration of approximately 0.1% - 45% of PLURONIC F-127 (BASF, Parsippany, NJ), otherwise known as poloxamer 407, in a ratio of approximately 1:2 to 1:4. A preferred final concentration of PLURONIC F-127 is 5% to 20%. The lecithin dissolved in isopropyl palmitate is added to 3 to 4 parts PLURONIC F-127 (for example, 20 ml lecithin in isopropyl palmitate plus 60-80 ml PLURONIC 127). Water, or any other agent known in the art may be added to effect a gel. Other PLURONICS may be used in the present invention. This mixture is called PLURONIC organogel or poloxamer organogel (PLO).

Other penetrating agents of the present invention associated with lecithin are phospholipids. Phospholipids that may be used in the present invention include, but are not limited to the following: phosphatidylethanolamine; phosphatidylcholineserine; dipalmitoylphosphatidyl choline; dimyristoylphosphatidylcholine, 1,2-dipalmitoyl-sn-glycerol phosphocholine; 1,2-dimyristoyl-sn-gly(3)phosphoglycerol; PHOSPHOLIPON 80™ 3-sn-phosphatidylcholine soya; PHOSPHOLIPON 90/90 G ™ 3-sn-phosphatidylcholine soya; PHOSPHOLIPON 90 H™ 3-sn-phosphatidylcholine soya hydrogenated; PHOSPHOLIPON CC™ 1,2-dicaproyl-sn-glycero (3) phosphocholine; PHOSPHOLIPON LC™ 1,2-dilauroyl sn-glycero (3) phosphocholine; PHOSPHOLIPON MC™ 1,2-dimyristoyl-sn-glycero (3) phosphocholine; PHOSPHOLIPON PC™ dipalmitoyl-sn-glycero (3) phosphocholine; PHOSPHOLIPON SC™ 1,2-distearoyl-sn-glycero (3) phosphocholine; PHOSPHOLIPON MG™ 1,2-dimyristoyl-sn-glycero (3) phosphoglycerol Na salt; PHOSPHOLIPON PG™ 1,2-dipalmitoyl-sn-glycero (3) phosphoglycerol Na salt; PHOSPHOLIPON SG™ 1,2-distearoyl-sn-glycero (3) phosphoglycerol Na salt; PHOSPHOLIPON G™ 3-(3-sn-phosphatidyl) glycerol soya; and PHOSPHOLIPON GH™ 3-(3-sn-phosphatidyl) glycerol soya hydrogenated. These PHOSPHOLIPON products are available from American Lecithin Co., Oxford, Connecticut.

Phospholipid gels are made by dissolving the desired phospholipid in any appropriate organic solvent. Organic solvents which may be used include but are not limited to methanol: chloroform (2:1 vol:vol) isopropyl palmitate, isopropyl myristate or ethanol. After the phospholipid is dissolved, 2 to 4 parts of PLURONIC 127 is added. Water may be added in an amount required to obtain the desired consistency. The organic solvents used for lecithin organogels, such as isopropyl palmitate and isopropyl myristate and others described in the present application are

also useful for preparation of gels containing natural and synthetic phospholipids.

The composition according to the present invention can be in the form of lotions, salves, creams, ointments, liposomes, micelles, sprays, gels, or administered in a pad or patch. The desired form is lotions, ointments and salves. Preferred embodiments include lecithin organogels, PLURONIC organogels, and phospholipid PLURONIC gels.

A gelling agent optionally may be added to the formulation. Gelling agents that are suitable for use in the present invention include, but are not limited to, carboxycellulose, alginates, polyacrylates, bentonite, gelatin, tragacanth, polyvinylpyrrolidone, polyvinyl alcohol, and polyoxyethylene/polyoxypropylene block copolymers.

A preservative, such as benzyl alcohol (concentration range of 0.05-5.0%, with a preferred concentration of 2.5%) or potassium sorbate, may be added to the composition. An antioxidant such as vitamin E tocopherol, proanthocyanidins, ascorbyl palmitate, ascorbic acid, or lipoic acid may be added to lecithin organogels. Other preservatives well known to those of ordinary skill in the art can be used in the composition, for example, 0.1% butylated hydroxide toluene (BHT), or 0.1% butylated hydroxide anisole (BTA). Other preservatives well known to those of ordinary skill in the art can be used in the composition.

Agents for improving the aroma of the formulation, defined herein as scents, can optionally be added to the composition. A desired aroma improving agent is honey almond oil (PCAA). Other aroma improving agents include, but are not limited to, avocado oil, sesame oil, castor oil, olive oil, grapeseed oil, clove oil, groundnut oil, corn oil, lemon oil, coconut oil, lime oil, hazelnut oil, jojoba oil, carthamus oil and wheatgerm oil. The oils can be added individually or in combination. It is to be understood that various fragrances and assorted floral scents may be optionally added to the

composition and are commercially available (PCAA). Stabilizers, antioxidants, preservatives, humectants, regreasing agents, solvents or auxiliaries can be added to improve stability and/or adhesiveness of the formulations. Cosmetic agents such as panthenol may also optionally be added to the formulation.

The method of the present invention includes topical administration of a pharmaceutically acceptable composition containing molecules in combination with a penetrating agent consisting of either lecithin organogel or poloxamer organogel optionally combined with phospholipid gels, any of which may be optionally combined with NDMS, cholesterol or ethanol. It is to be understood that either lecithin organogel, phospholipids or poloxamer organogel and/or cholesterol may be optionally combined with NDMS to act as penetrating agents. In one embodiment, lecithin organogel, phospholipids and poloxamer organogel and/or cholesterol may be used optionally in combination with NDMS to act as penetrating agents. The composition of the present invention can be administered topically either once daily or several times per day depending upon the nature and severity of the condition being treated. In another embodiment bromelain and capsaicin may be combined with NDMS and/or lecithin organogel, phospholipids and/or poloxamer organogel for the treatment of inflammation. The anti-inflammatory composition of the present invention can be administered topically either once daily or several times per day depending upon the nature and severity of the inflammatory condition being treated.

Preferably, the anti-inflammatory composition of the present invention is applied topically at the site of the inflammation. For example, for osteoarthritis of the knee, the anti-inflammatory composition of the present invention is applied topically around the knee by rubbing the composition on the skin. Typically, the anti-inflammatory composition of the present invention is applied in widths of approximately 1.5

cm, 2.5 cm or 3.5 cm. If the composition is applied to a painful joint, it can be applied directly on one side of the joint or can be evenly rubbed around the entire joint. If severe pain exists, it is helpful to apply an equal amount of the anti-inflammatory composition of the present invention on the anterior and posterior surface between 1 and 4 times daily for 2 weeks. The anti-inflammatory composition of the present invention should be applied until the pain subsides. The amount of the composition that is applied to the skin is not critical to the invention. It is important that the composition be thoroughly rubbed into the skin.

Although not wanting to be bound by the following hypothesis, it is believed that the composition of the present invention causes at least a portion of a molecule, for example bromelain, to be transdermally delivered to the site of the inflammation. In this manner, bromelain can exert its anti-inflammatory effect at the site of inflammation. In addition, the present invention causes more capsaicin to be delivered to the inflammatory site thereby relieving pain presumably by inhibiting release of substance P.

While not wanting to be bound by the following statement, it is believed that bromelain may increase levels of cyclic AMP which may cause a down-regulation of interleukin-1. This reduction in interleukin-1 may affect the processing of hapten by Langerhans cells in the skin. While not wanting to be bound by the following statement, it is believed that reduction of interleukin-1 can decrease production of collagenase and prostaglandin by synovial fibroblasts and articular chondrocytes, as well as production of interleukin 8. It is also believed that the elevation of cyclic AMP in psoriasis helps to stabilize the activity of keratinocytes by down regulating colony stimulating factor and interleukin-1.

A variety of other molecules may be administered for transdermal transport using the composition and method of

the present invention. The molecules include, but are not limited to molecules that may be classified in the following categories; enzymes, coenzymes, protease inhibitors, proteins, peptides, amino acids, hormones, growth factors, interleukins, immunoglobulins, cytokines, monokines, drugs, vitamins, plant extracts, lipids, plasmids, nucleic acids, including but not limited to ribonucleic acids and deoxyribonucleic acids, nucleotides, neurotransmitters, neurotransmitter agonists and antagonists, steroids, lipids, fatty acids, carbohydrates and antifungal agents. As used herein, the word "molecule" is used to describe any compound or substance, such as those compounds or substances that fall into the categories described in this paragraph. The word "molecule" is not limited to a single molecule or to any number of molecules.

The present invention and method permits the transdermal transport of the different types of molecules recited above for the treatment of a wide variety of conditions. Some of these conditions include but are not limited to the following; tendonitis, desmitis, bursitis, thrombophlebitis, cellulitis, muscular inflammation, myalgia, arthritis including osteoarthritis and rheumatoid arthritis, synovitis, carpal tunnel syndrome, venomous and non-venomous insect bites, onchomycosis of the toenail and fingernail, fungal diseases of the skin and scalp, and joint strain and sprain. For example, autoimmune phenomena in joints could be treated with antiinflammatory agents such as polyclonal or monoclonal antibodies designed to bind autoimmune antibodies. A variety of immunomodulatory molecules may be transdermally transported with the present invention including but not limited to interleukins, immunoglobulins, cytokines, and monokines. The effective amount of molecule selected to treat a particular condition will depend on the specific condition being treated.

In another embodiment, the present invention may be placed in the form of a skin patch or ointment for use to

transdermally transport one or multiple antigens used for immunization, thereby avoiding the use of injections and needles. In a different embodiment a skin patch may be placed on the lower back, neck, shoulder or other site for relief of pain. In a different embodiment, non-steroidal hormones such as natural or recombinant pituitary hormones or hypothalamic releasing and inhibiting factors may be transdermally transported with the present invention to affect a variety of conditions, including but not limited to growth, reproduction, inflammation, metabolism, bone metabolism, electrolyte balance, water balance, glucose homeostasis, diabetes, production of blood cells, and hypertension.

In still another embodiment, the present invention may be used to transport growth factors, including but not limited to, fibroblast growth factor, epidermal growth factor, nerve growth factor, and growth factors that affect granulocytes, macrophages, and reticulocytes.

It will be appreciated that other embodiments and uses will be apparent to those skilled in the art and that the invention is not limited to these specific illustrative examples.

Example I

Bromelain-Capsaicin Topical Composition

A bromelain-capsaicin topical composition (Bromelain 7.5%/PLURONIC organogel) is prepared as follows:

Bromelain Powder	7.5 g
Capsaicin	25 mg
Honey Almond Oil	2 mL
Lecithin-Isopropyl Palmitate	20 mL
20% PLURONIC Mixture	80 mL

Lecithin was prepared by dissolving 10 g of soy lecithin granules (PCAA, Houston TX) in 10 mL of isopropyl palmitate. 1% benzyl alcohol was added as a preservative.

The mixture was stirred periodically for 24 hours until the soy lecithin dissolved. The 20% PLURONIC mixture was prepared by dissolving 16 g PLURONIC® 127 (BASF, Parsippany, N.J.) in 80 mL of distilled water. Then PLURONIC organogel was made by adding the 20% PLURONIC mixture to the lecithin-isopropyl palmitate mixture and stirring.

The Bromelain 7.5%/PLURONIC organogel was prepared by mixing the bromelain powder with the lecithin organogel and honey almond oil until a smooth mixture is prepared. The 20% PLURONIC mixture was added at 4 parts to 1 part lecithin-isopropyl palmitate and mixed until a gel formed. The composition was stored at room temperature.

Example II

Bromelain-Capsaicin Roll-on Formulation

A bromelain-capsaicin roll-on formulation is prepared as follows:

Bromelain	7.5 g
Capsaicin	25 mg
n-decylmethyl sulfoxide	500 mg
Ethoxydiglycol	5 mL
Distilled water	50 mL
98% lecithin-isopropyl palmitate	20 g
PLURONIC 127 solution (10-20%)	30 mL
Honey almond oil	2 mL

The bromelain powder was stirred into water. NDMS was added and stirred continuously for 2 hours followed by addition of the honey almond oil. Next lecithin organogel or poloxamer organogel was added.

Example III

Transport of Albumin Across the Stratum Corneum Using PLURONIC Organogel

Bovine albumin (molecular weight 66,000 Daltons) conjugated to fluorescein isothiocyanate (FITC from Sigma Chemical Co., St. Louis, MO) was incorporated at a concentration of 7.5% into a mixture of PLURONIC organogel. A rat was anesthetized with isoflurane, the back was shaved with an oster blade, and the skin wiped with 90% ethyl alcohol. The albumin-FITC was applied to the skin in the auricular area. FITC was injected intradermally in other locations on the skin of the rats as a positive control. Albumin-FITC conjugate was mixed in hand cream in the absence of PLURONIC organogel and applied to the skin of the rat as a negative control. The hand cream employed was Rite Aid extra strength skin care lotion and did not contain any penetrating agents. The hand cream contained dimethicone as the active ingredient, and also contained water, glycerin, stearic acid, C_{11-13} isoparaffin glycol stearate, petrolatum, glyceryl stearate, triethanolamine, zinc oxide, cetyl alcohol, potassium cetyl phosphate, carbomer 934, cetyl acetate, acetylated lanolin alcohol, stearamide AMP, magnesium aluminum silicate, methylparaben, and disodium EDTA.

Two hours after application of these compounds, skin biopsies approximately 6 mm in diameter were removed, sectioned at 4 microns (μm) in a cryostat, and placed on glass slides. One specimen was fixed in alcohol, dehydrated through absolute alcohol and a xylene substitute and coverslipped with Permount. The other specimen was air dried and not coverslipped. The slides were examined by a pathologist in an Olympus BH-2 microscope with a reflected light fluorescent attachment. In the albumin-FITC test condition, higher than the background levels of fluorescence exhibited by the negative controls were observed in the intercellular spaces of the epidermis. Fluorescence in the epidermis of test samples was

lower than the intense fluorescence in the positive controls after intradermal injection of FITC-albumin conjugate. These results are consistent with FITC-labeled bovine albumin percolating between epidermal keratinocytes in the presence of the PLURONIC organogel.

Example IV

Transport of Caprine Immunoglobulin Across the Stratum Corneum Using PLURONIC Organogel

A PLURONIC organogel was prepared (2 parts PLURONIC 127, 2 parts lecithin-isopropyl palmitate mixture plus 5% cholesterol) with 93% phosphatidyl choline (Phospholipon 90, American Lecithin Co., Oxford, Connecticut) and 10% caprine immunoglobulin (Goat-IgG, approximate molecular weight of 150,000 Daltons) conjugated to FITC (SIGMA) was added to this gel. The auricular area was shaved on both sides of two rats and the skin cleaned with alcohol. About 0.5 mL of the PLURONIC organogel was applied to the right auricular skin. The controls received hand cream applied to the left auricular skin containing caprine IgG-FITC conjugate. Three hours later, the auricular skin was thoroughly cleaned with alcohol and soap and biopsies were removed. Examination of the samples from the right auricular skin showed fluorescence in the dermis while the controls did not exhibit fluorescence in the dermis.

Example V

Collagenase Denaturation of Dermal Collagen in Rat Skin

Collagenase with a molecular weight of about 102,500 Daltons (PCAA, Houston, Texas) was incorporated at 10% into a gel containing 40% phosphatidylcholine (Lecithin, Sigma Chemical Co., St. Louis, MO.) and 10% PLURONIC F-127. The collagenase and phosphatidylcholine gel was dissolved in an equal amount of isopropyl palmitate and four parts PLURONIC 127 (20% solution) and 2 mL of 10% calcium

chloride. Two rats were shaved in the cervical region and the gel was applied twice daily for 3 days. The animals were anesthetized with isoflurane and skin biopsies were obtained at the site of gel application and at other sites.

In one experiment, histopathological analysis of the samples indicated intercellular edema and superficial fragmentation. The edema and fragmentation were noted immediately subjacent to the epithelium. The collagen bundles appeared more widely separated and were thin, fine, and pale staining. Other biopsy sites untreated with gel containing collagenase revealed normal cellular morphology and tissue architecture.

Eighteen days following the initial biopsy new biopsies were obtained. One specimen still demonstrated thin, fine and pale staining collagen fibers subjacent to the epithelium. The second experimental biopsy was normal in appearance probably indicating that this area of the skin underwent less severe degeneration resulting in faster collagen regeneration. Further examination of the original biopsy stained with Mallory trichrome revealed that one of the specimens exhibited a sharply demarcated subepidermal area with diminished staining intensity of collagen. The area of diminished staining was parallel to the epidermal surface. The immediately overlying epidermis was unremarkable. The results indicate that collagenase was transported across the stratum corneum to the subepidermal space where it exhibited enzymatic activity.

Example VI

Transdermal Transport of the Macromolecule Bromelain: Demonstration of Protease Action of Bromelain

Approximately 100 mg of Eastern cottonmouth (*Agkistrodon piscivorus piscivorus*) venom (SIGMA, St. Louis, MO) was dissolved in 20 mL of physiological saline solution (0.9% saline) providing a solution of 5 mg/mL. This

was further diluted by adding 0.85 mL of 0.9% saline to 0.15 mL of the 5 mg/mL solution, providing 0.75 mg/mL. About 0.05 mL of this solution was injected intradermally in the control and experimentally treated skin. Skin was experimentally treated by applying about 0.5 mL of a 7.5% bromelain in PLURONIC organogel to a shaved area of back skin of a rat. Negative controls received 7.5% bromelain mixed in hand cream (Rite aid) applied to a shaved area of skin in the anterior lumbar region of the back. After about 45 minutes, the necrotic areas were measured with an electrocardiogram caliper.

Five mice were treated with 0.25 mL 8% bromelain in PLURONIC organogel and 5 mice were treated with 0.25 mL hand cream with 8% bromelain as a control. Copperhead venom (0.05 mL of a 0.9 mg/mL solution) was injected intradermally to all mice under isofluorane anesthesia. Five experimental mice survived 48 hours whereas the controls died within 24 hours. The results demonstrate efficacy of transdermally transported bromelain to partially inactivate the active components of the venom.

Example VII

Transdermal Transport of the Macromolecule Bromelain: Demonstration of Protease Action of Bromelain to Denature Destructive Capability of Snake Venom

Ten mice were treated with about 0.25 mL bromelain in PLURONIC organogel (experimental) on one location of the skin of the back. The same mice received bromelain in hand cream (control) at another location on the skin of the back. At each of these experimental and control sites, approximately 0.05 mL of a solution of copperhead venom (100 mg/ 20 mL of physiological saline. From this solution 0.15 mL was diluted in 0.85 mL physiological saline).

Table I

Size of Necrotic Regions (mm in diameter) of Control and Experimental Cutaneous Sites

Control	Experimental
11	5.5
18	10
7	5
10	5.5
8	4.75
21	15
22	14
16	11
19	12
24	15
Σ156 Total Area	Σ 97.8 Total Area

The results demonstrate approximately 37% less necrosis in experimental treatment sites showing partial amelioration of the destructive effects of snake venom in the presence of transdermally transported bromelain by PLURONIC organogel.

Example VIII

Demonstration of Transdermal Transport of a Reduced Amount of the Macromolecule Bromelain Combined with Cholesterol: Demonstration of Protease Action of Bromelain to Denature Destructive Capability of Snake Venom Potentiated by Cholesterol

The same procedure was employed as in Example VII using cottonmouth venom with the following exceptions: bromelain was reduced to 3%, and 10% cholesterol was added to the gel made from phospholipon C and PLURONIC 127 organogel. Five rats were anesthetized and prepared as

described in Example VI. The right forearm (experimental) was treated with 0.5 mL bromelain in PLURONIC organogel with cholesterol and the left forearm was used as a control with 0.5 mL of hand cream (Rite aid) containing 3% bromelain. About 45 minutes later, all gel and cream were removed carefully and 0.05 mL venom injected intradermally at these experimental and control sites. Approximately 45 minutes later the necrotic areas were measured with an electrocardiogram caliper.

Table II

Size of Necrotic Regions (mm in diameter) of Control and Experimental Cutaneous Sites

Control	Experimental
8	5
9	5
10	6
10	6
11	8
Σ48 Total Area	Σ30 Total Area

The results indicate a 38% decrease in the total necrotic area of sites receiving bromelain application in the presence of PLURONIC organogel and cholesterol, suggesting that transdermal transport of reduced amounts of the protease bromelain inhibited the extent of the venom-induced necrosis.

Example IX

Transdermal Transport of Bromelain for the Relief of Pain

Inflammation of the distal interphalangeal joint is a painful condition. Bromelain (approximately 7.5%) in PLURONIC organogel was applied in a pea sized drop to painful distal interphalangeal joints in seven human volunteers

(age range 18 to 75 years) who reported pain in these joints. After application of the bromelain in PLURONIC-lecithin organogel, all volunteers reported relief from the pain for a period of about 8 to 12 hours. Complete relief from pain was reported by 5 volunteers while 2 reported significant but not complete relief from pain.

These results demonstrate that bromelain, a molecule of about 33,000 Daltons molecular weight, was effectively transported through the skin and alleviated pain, perhaps by its action on subcutaneous nerve endings, possibly pain afferents.

Example X

Transdermal Transport of Bromelain Inhibits Histamine-Induced Inflammation and Flea Antigen-Induced Inflammation

Topical application of about 0.25 mL of an 8% solution of bromelain in PLURONIC-lecithin organogel was followed by thoroughly rubbing it into the skin of the arm of a human volunteer. Next, 0.05 mL of a solution of histamine (5 mg/mL) was injected intradermally into the arm at the site of bromelain application. A second injection was administered in the forearm, a site that was not treated with bromelain. Ten trials employing six different gels revealed a 45% reduction in the size of the wheal produced by histamine injection at the site of bromelain application compared to the site without bromelain application.

In a second experiment, topical application of about 0.25 mL of an 8% solution of bromelain in PLURONIC organogel was followed by thoroughly rubbing it into the skin of the arm of a human volunteer. Next, 0.05 mL of flea antigen (Greer Labs, Lenoir, NC) diluted 1 to 100 in physiological saline solution was injected intradermally into the arm of a human volunteer at the site of bromelain application. A second injection was administered in the forearm, a site that was not treated with bromelain.

Table III

Effect of Transdermal Bromelain Application on Flea-antigen Induced Wheal Formation (wheal size (diameter in mm))

Control	Experimental
19.5	13
17	11
22	12
19	13
23	11
Σ100.5 Total Area	Σ60 Total Area

Wheal size was determined by measuring the greatest diameter with an EKG caliper. The results indicated a 39% decrease in the wheal size at the site of bromelain application, suggesting that transdermal transport of the protease bromelain inhibited the degree of inflammation.

Example XI

Formation and Testing of Phospholipid Pluronic Organogel: Demonstration of Transdermal Transport Using Phospholipid Organogel

Semisynthetic phospholipids may also be utilized for gel preparation. A phospholipid organogel was made by dissolving 1.94 g of Phospholipon CC (1,2 dicaproyl-sn glycerol 3 - phosphocholine) (American Lecithin Co., Oxford, CT) in about 1.94 g of isopropyl myristate, 0.5 ml ethanol, 4 ml of PLURONIC 127 (20% solution) and 4 ml of deionized H_20. Bromelain was used to evaluate protease activity against fescue grass extract used as the allergen (0.1 ml extract + 9.9 ml H_2O) (Greer Lab, Lenoir, N.C.). Bromelain (400 mg) was added by mechanical stirring until a gel was formed (experimental sample). Next, 400 mg of bromelain was added

separately to a hand cream (control) to achieve the same final concentration as the experimental sample. In order to test the penetration capability of the phospholipid organogel compared to the hand cream, a rat was anesthetized and the back carefully shaved and cleaned with 70% isopropyl alcohol. Approximately 0.5 ml of the phospholipid organogel was applied to the caudal aspect of the skin of the back and about 0.5 ml of the control sample was applied to the rostral lumbar skin of the back. About 1.5 hours later the experimental phospholipid gel and the control cream were removed from the skin. Approximately 0.05 ml of an aqueous 1:100 dilution of Fescue extract (Greer Lab., Lenoir, NC) as allergen was injected intradermally into the sites exposed to either the experimental or control compositions. The allergen injections produced wheals which were measured three hours after injection. The average size of the wheals at control sites was 40% larger than the wheals at sites treated with the phospholipid organogel. These results demonstrate that bromelain was transdermally transported at sites treated with phospholipid organogel. The protease nature of the transdermally transported bromelain partially deactivated the allergen, resulting in reduced wheal diameter at sites treated with phospholipid organogel compared to sites treated with bromelain in hand cream.

Example XII

Preparation of Phospholipid Organogels

Approximately 98-99% pure phospholipids were dissolved in isopropyl palmitate (isopropyl myristate may also be used) with 3-5 ml 98% ethanol and allowed to stir over night using a mechanical stirrer. Gels were made by titrating the composition with sterile water (or saline): water was added until the desired viscosity was achieved. Optionally, instead of using water or saline for titration, PLURONIC 127 may also be used for making a gel. If PLURONIC 127 is

used, then it is used in a proportion of 1:4, i.e., one part phospholipid and four parts PLURONIC 127. The use of PLURONIC 127 generally reduces the amount of phospholipids that are utilized, consequently, penetration of the dermis or other desired targets could be affected.

Example XIII

Treatment of Pain Associated with a Knee Injury

Severe knee injury, caused by an automobile accident, was treated in a 68 year old woman using a composition consisting of a PLURONIC organogel made with lecithin, isopropyl palmitate, bromelain and capsaicin. The knee injury caused the patient severe discomfort and pain, and also limited flexion and extension. A PLURONIC organogel containing 7.5% bromelain and 0.25% capsaicin was made. A 60% lecithin powder was dissolved gram for gram with isopropyl palmitate and 4 parts PLURONIC 127 (20% solution) to make a gel. Following topical treatment of the knee with this composition, the patient reported 90% reduction in pain.

Example XIV

Use of PLURONIC Lecithin Organogel for Transdermal Delivery of Methotrexate, a Polar Molecule

Methotrexate is a highly effective drug administered orally for rheumatoid arthritis, but it is not used at the initial stages of the disease due to its toxicity.

One part of lecithin isopropyl palmitate mixture is added to four parts of PLURONIC 127 (20% solution). Methotrexate was added to make a 5% methotrexate gel with a 20% lecithin dissolved in equal parts of isopropyl palmitate. 0.5 ml of the gel was applied to the shaved knee of a 60 lb. dog. Three hours later synovial fluid and blood samples were drawn and submitted to Roche Biomedical, Burlington, VT, for analysis. The methotrexate concentration in the blood sample was less than 0.01 μmol/l. The methotrexate

concentration in the synovial sample was 0.07 µmol/l which was in the low therapeutic range. The same dog was given 2.5 mg orally and 3 hrs. later blood was drawn and the methotrexate concentration was 0.76 µmol/l. The methotrexate concentration in the synovial fluid was 0.42 µmol/1.

A 120 lb dog was treated with 8 ml of methotrexate on the knee and the synovial fluid and blood samples were drawn 24 hrs. later. The blood level of methotrexate was 0.07 µmol/l and the synovial level of methotrexate was 0.17 µmol/l.

Treatment	Blood Sample	Synovial Fluid Sample
0.5 ml MTZ gel on shaved knee, samples drawn 3 hours later	<0.01 µmol/l	0.07 µmol/l
2.5 mg orally, samples drawn 3 hours later	0.76 µmol/l	0.42 µmol/l
8 ml MTZ gel on shaved knee, samples drawn 24 hours later	0.07 µmol/l	0.17 µmol/l

These data suggest that rheumatoid arthritis in children and animals could be treated more aggressively without a concern for toxicity that precludes its use near the onset of the disease.

Example XV

Transdermal Transport of Collagenase with a Pluronic Lecithin Organogel

Phospholipon 80 (American Lecithin Co., Oxford, CT.) was dissolved in an equal weight (gram for gram) of isopropyl myristate and 30 ml of PLURONIC 127 (20% solution). Deionized water (10 ml) was added to 10 ml of the phospholipid PLURONIC mixture. As the gel was forming, 10 mg collagenase and 2 ml $CaCl_2$ was added. A rat was treated as previously and biopsied. Five days later the experimental animal was rebiopsied 3-5 mm on either side of the first site. Under normal conditions a distinct basement membrane is generally not observable at the sites of separation, but where the basement membrane persists it is present bordering the basal aspect of the basal epithelial cells. Collagenase has also been successfully transported with lecithin organogel using lecithin obtained from egg yolk. Similar results were obtained.

Example XVI

Preparation of Lecithin Organogel

Approximately 95% pure lecithin may be dissolved in isopropyl palmitate or isopropyl myristate on a weight basis of 1 g of lecithin per about 0.5 to 1.5 g of isopropyl palmitate or isopropyl myristate and/or ethanol. The preferred ratio of lecithin to these solvents is about 1 g to about 0.75 g to 1 g. Next ethanol (98%) may be added while stirring at 80° C until the alcohol is boiled off. Water is then added with stirring at approximately 20 to 40% with a preferred concentration of about 30%.

A penetration enhancer of the present invention is PLURONIC F-127 (BASF, Parsippany, NJ) which permits use of lecithins of lesser purity than those required in formation of lecithin organogels as taught by Willimann et al. PLURONIC F-127 is employed at concentrations of about 0.1% to 45% in a

ratio PLURONIC to lecithin of about 1:0.5 to 1:6.0. A preferred final concentration of PLURONIC F-127 is 5% to 20% in a ratio of PLURONIC to lecithin of 1:2 to 1:4. Lecithins of concentrations of approximately 5% to 90% are first dissolved in isopropyl palmitate, isopropyl myristate and/or 98% ethanol. The addition of four parts of PLURONIC F-127 (20% solution) to the dissolved lecithin produces a cost effective gel. In addition, water, carboxyethyl cellulose, carboxymethyl cellulose, other PLURONICS, and other agents known to one skilled in the art may be used. These mixtures are known PLURONIC organogels or poloxamer organogels.

Example XVII

Cholesterol as a Penetration Enhancer

Water dispersible lecithin (60% concentration-Precept 8140 Central Soya, IN) is added to an equal amount of deionized water in a ratio of approximately 10 g lecithin to 10 g deionized water. These reagents are mechanically stirred. Optionally, ethoxydiglycol may be added in a range of about 5% to 35% with a preferred concentration of approximately 10%. Next, cholesterol is added to the lecithin in a desired molar proportion and heated to approximately 65° C. Addition of 98% ethanol to the mixture accelerates fusion of cholesterol in the micelle and acts as a penetration enhancer. Increasing the temperature to 80° C will boil off excess ethanol. A desired final concentration of ethanol, however, is 3% to 8% in order to enhance penetration. Once a homogenous mixture is obtained, PLURONIC F127 (20% concentration) is added in a ratio of approximately 3:1 to 4:1 to effect gel formation. A composition prepared by this method may be advantageous in situations where chronic use might provoke hypersensitivity reactions to isopropyl palmitate, isopropyl myristate or ethanol.

Cholesterol may be used in an identical manner in the preparation of other organogels as taught in the present

invention. These organogels include lecithin organogels, phospholipid gels, PLURONIC organogels and PLURONIC-phospholipid gels. The use of cholesterol provides increased stability and penetration of the gels. The relative concentration of cholesterol to phospholipid may be determined by one skilled in the art and would involve a consideration of the molecules to be transported as well as the phospholipids employed in forming the phospholipid gel. Molar concentrations of cholesterol to phospholipid in cholesterol phospholipid gels would be approximately 0.1:1.5. In another embodiment of cholesterol as a penetration enhancer, approximately 7.66 g of water dispersible lecithin at 60% concentration (Precept 8140, Fort Wayne, IN) is added to 7.5 ml of deionized water and 2.5 ml of ethoxydiglycol followed by mechanical stirring. Upon uniform dispersion of the lecithin, 1.9 g of cholesterol (0.5 M cholesterol:1 M lecithin) is added and stirred at about 65° C. Cholesterol disperses and is incorporated into the micelle. Optionally, 2-10 ml of 98% ethanol may be added to accelerate this process, however, in this case the temperature must be increased to about 80° C.

The excess ethanol is then boiled off so that the final concentration of ethanol in the mixture is approximately 3 to 8 %. Next, PLURONIC 127 (20%) is added at a ratio of approximately 3 to 4 part of PLURONIC to 1 part of the lecithin/cholesterol mixture. After the temperature of this mixture falls to about 25° C, 4 g of bromelain is then thoroughly blended into the mixture using a stirring apparatus. A control bromelain cream is made by adding 400 mg to 9.6 g of hand cream.

Next 1 ml of bromelain PLURONIC organogel was applied to the medial proximal forearm and a similar amount of the control cream was applied to the distal medial forearm. About 1.5 hours later, 0.05 ml of a 1:100 dilution of a fescue antigen (Greer Lab., Lenoir, NC) was injected intradermally

after each site was carefully cleaned. The resulting wheals were 30% larger in control areas when measured 3 hours after injection. These results indicate that cholesterol may be beneficial in transdermally transporting bromelain in the absence of an organic solvent since wheal sizes in experimental areas were smaller than those observed in control areas.

Example XVIII

Transdermal Transport of Collagenase Using an Egg Yolk Lecithin, PLURONIC, Isopropyl Myristate Composition

Egg yolk lecithin at approximately 98% concentration (USB Cleveland, OH) was dissolved in about 100 g of isopropyl myristate. Ten ml of this mixture was added to about 10 ml of PLURONIC F127 (20% concentration), 20 ml of water, 10 mg collagenase, and 1 ml $CaCl_2$. A control sample was prepared by dissolving collagenase in hand cream until the same final percentage of collagenase was obtained as used in the experimental cream. The backs of rats were shaved in the anterior lumbar region and the experimental composition was applied in a volume of approximately 0.5 ml twice at 12 hour intervals. The next morning the animals were anesthetized and vesicles were evident at biopsy which were not present in controlled treated animals. Histopathological analysis revealed extensive subepidermal bulla formation at sites of application of the experimental composition containing collagenase. In the center of these biopsy specimens, the epidermis was separated from the subjacent dermis by a wide clear space. Collagen was slightly paler in appearance than the collagen subjacent to the clear space and was also less distinct and slightly paler than the collagen subjacent to the intact epithelia. Biopsies from control animals were described as normal in histologic appearance. The lesions observed in biopsies from experimental animals resembled those observed in bullous and vesicular diseases caused by loss of the structural integrity of

the basement membrane zone. These results indicate the utility of combining phospholipids in PLURONIC gels.

It should be understood that the foregoing relates only to preferred embodiments of the present invention and that numerous modifications or alterations may be made therein without departing from the spirit and the scope of the invention as set forth in the appended claims.

CLAIMS

I claim:

1. A composition for transdermal transport of molecules comprising an effective amount of a pharmaceutically effective penetrating agent.

2. The composition of Claim 1, wherein the pharmaceutically effective penetrating agent is lecithin organogel, phospholipid gel, poloxamer phospholipid gel, or poloxamer lecithin organogel optionally combined with *n*-decylmethyl sulfoxide, ethoxy diglycol, ethanol or cholesterol.

3. The composition of Claim 2, further comprising molecules selected from the group consisting of enzymes, elastase, coenzymes, protease inhibitors, proteins, collagen, receptors, peptides, amino acids, hormones, hormone agonists and antagonists, growth factors, interleukins, immunoglobulins or fragments thereof, cytokines, monokines, drugs, vitamins, plant extracts, lipids, plasmids, nucleic acids, nucleotides, neurotransmitters, neurotransmitter agonists and antagonists, steroids, lipids, fatty acids, carbohydrates, anticancer agents, antibacterial agents, antifungal agents, antiprotozoal agents, antihelminthic agents, antiviral agents, capsaicin, bromelain, methotrexate, and molecules that affect signal transduction systems.

4. The composition of Claim 3, further comprising scents, gelling agents, compounding agents, preservatives, stabilizers, skin moisturizers, humectants, regreasing agents, cosmetic agents, solvents or auxiliaries or ultraviolet blockers.

5. A method for transdermally transporting molecules comprising topical application of an effective amount of a pharmaceutically effective penetrating agent in combination with a molecule or more than one molecule.

6. The method of Claim 5, wherein the pharmaceutically effective penetrating agent is lecithin organogel, phospholipid gels, poloxamer phospholipid gels, or poloxamer lecithin organogel optionally combined with *n*-decylmethyl sulfoxide, ethoxy diglycol, ethanol or cholesterol.

7. The method of Claim 5, wherein the pharmaceutically effective penetrating agent is a gel, cream, spray, rinse, ointment, salve, balm, liposome, time release vehicle, micelle, skin patch or pad.

8. The method of Claim 5, wherein the molecule or molecules are selected from the group consisting of enzymes, elastase, coenzymes, protease inhibitors, proteins, collagen, receptors, peptides, amino acids, hormones, hormone agonists and antagonists, growth factors, interleukins, immunoglobulins or fragments thereof, cytokines, monokines, drugs, vitamins, plant extracts, lipids, plasmids, nucleic acids, nucleotides, neurotransmitters, neurotransmitter agonists and antagonists, steroids, lipids, fatty acids, carbohydrates, anticancer agents, antibacterial agents, antifungal agents, antiprotozoal agents, antihelminthic agents, antiviral agents, capsaicin, bromelain, methotrexate, and molecules that affect signal transduction systems.

9. A method for administering molecules to treat a condition comprising topical administration of an effective amount of a pharmaceutically effective penetrating agent in combination with the molecules to treat the condition.

10. The method of Claim 9, wherein the pharmaceutically effective penetrating agent is lecithin organogel, phospholipid gel, poloxamer phospholipid gel, or poloxamer lecithin organogel optionally combined with *n*-decylmethyl sulfoxide, ethanol or cholesterol.

11. The method of Claim 9, wherein the condition is neuralgia, myalgia, fibromyalgia, arthritis, rheumatoid arthritis, osteoarthritis, sprains, strains, bursitis, tendonitis, myositis, carpal tunnel syndrome, chondromalacia, eczema, infections, bites, psoriasis, acne, eczema, dermatitis, solar elastosis, thrombi, phlebitis, hematoma, fungal disease, or atopy.

12. The method of Claim 9, wherein the condition is pain, or deficiencies or imbalances of the immune system, integumentary system, endocrine system, reproductive system, cardiovascular system, musculoskeletal system, nervous system, digestive system, and respiratory system.

13. The method of Claim 11, wherein the fungal disease affects the skin or scalp.

14. The method of Claim 13, wherein the fungal disease is onchomycosis of the toenail or fingernail.

15. The method of Claim 9, wherein the pharmaceutically effective penetrating agent is a gel, cream, spray, rinse, ointment, salve, balm, liposome, time release vehicle, micelle, skin patch or pad.

16. The method of Claim 9, wherein the pharmaceutically effective penetrating agent is administered once or several times per day.

17. The method of Claim 9, wherein the condition is rheumatoid arthritis and the molecule is methotrexate.

18. The method of Claim 9, wherein the condition is psoriasis and the molecule is methotrexate.

19. The method of Claim 9, wherein the condition is solar elastosis and the molecule is elastase.

20. The method of Claim 9, wherein the molecule is selected from the group consisting of enzymes, coenzymes, protease inhibitors, proteins, collagen, receptors, peptides, amino acids, hormones, hormone agonists and antagonists, growth factors, interleukins, immunoglobulins or fragments thereof, cytokines, monokines, drugs, vitamins, plant extracts, lipids, plasmids, nucleic acids, nucleotides, neurotransmitters, neurotransmitter agonists and antagonists, steroids, lipids, fatty acids, carbohydrates, anticancer agents, antibacterial agents, antifungal agents, antiprotozoal agents, antihelminthic agents, antiviral agents, capsaicin, bromelain, elastase, methotrexate, and molecules that affect signal transduction systems.

INTERNATIONAL SEARCH REPORT

International application No.
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A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12N 9/50; A61F 13/00
US CL :435/219; 424/449; 514/570

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/219; 424/449; 514/570

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,264,219 A (GODBEY et al) 23 November 1993, see entire document.	1-20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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Date of the actual completion of the international search	Date of mailing of the international search report
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